Contents list available at CBIORE journal website

International Journal of Renewable Energy Development

Journal homepage: https://ijred.cbiore.id

Research Article

Technology and equipment for bioethanol producing from plant waste

Dmitry Prosvirnikov [a], Denis Tuntsev [a], Yuliya Kulikova [b*], Olga Babich [b]

[a]*Chemical Cybernetics Department, Institute of Food Engineering and Biotechnology, Kazan State Technological University, Kazan, Russia*

[b]*Institute of Living Systems, IKBFU, Kaliningrad, Russia*

Abstract. This study addresses the critical challenge of utilizing waste from agricultural industries by developing an efficient technology for its conversion into bioethanol. The research focuses on the application of steam explosion treatment as a pre-activation step for lignocellulose biomass, specifically pine wood and wheat straw. The novelty of the work lies in obtaining new fundamental data on the acid and enzymatic hydrolysis of steam-exploded materials and optimizing the subsequent biochemical conversion process to produce an alternative energy resource. The raw materials were subjected to steam explosion activation at saturated steam temperatures of 165 °C and 210 °C. Component analysis revealed that this pre-treatment effectively removes hemicelluloses and pentose sugars, which are not fermented by standard yeasts, while increasing the specific surface area and reactivity of the lignocellulose matrix. Acid hydrolysis of activated pine wood and enzymatic hydrolysis of activated wheat straw were investigated to produce hydrolysates rich in hexose monosaccharides. The results demonstrated that steam-explosive activation significantly enhances the hydrolysis rate and yield of reducing substances (RA). Activated pine wood was maximally converted to RA 1.7-2.5 times faster than untreated wood. In addition, activation at 210 °C allowed us to obtain hydrolysates with a minimum content of pentose, which in this study acted as inhibitors of the fermentation process. Subsequent anaerobic fermentation of these optimized hydrolysates using *Saccharomyces cerevisiae* achieved high ethanol yields. Specifically, steam-explosive activation of pine wood at 210 °C enabled the production of up to 0.26 kg (0.33 L) of ethanol per 1 kg of activated raw material, representing 36.7% of the RA. For wheat straw activated at the same temperature, enzymatic hydrolysis yielded up to 0.172 kg (0.218 L) of ethanol per 1 kg of activated straw. The study concludes that steam explosion is a highly effective pre-treatment method, facilitating the production of high-quality hydrolysates for efficient bioethanol production, thereby contributing to sustainable energy development and waste valorization.

Keywords: bioethanol, steam explosive activation, hydrolysis, enzymatic hydrolysis, fermentation, agricultural waste.

Received: 10th Nov 2025; Revised: 5th January 2026; Accepted: 29th January 2026; Available online: 8th Feb 2026

1. Introduction

The problem of utilization of plant waste, the formation of a significant amount of which in the logging, woodworking and agricultural industries is caused by the irrational use of plant, in particular wood, raw materials at enterprises of the corresponding profile, is very relevant. Along with this, in the modern world, every year there is a growing demand for new environmentally friendly technologies for processing plant materials into products such as cellulose and microcrystalline cellulose, as well as bioenergy resources for the production of fuels and chemical products (Burdukov *et al.* 2018, Sititsyn *et al.* 2021, Keldiyorova *et al.* 2018).

In the face of the threat of depletion of natural resources, the search for alternative energy sources is relevant. The real way to solve this problem is the production of biological types of energy resources - bioethanol, biomethane, biohydrogen (Lakina *et al.* 2018, Tsegaye *et al.* 2019). The correctness of this path has been confirmed by many years of world practice in Europe and the United States. These countries are at the stage of transition from the production of first-generation biofuels (bioethanol - from sugar and grain; biodiesel - from oilseeds) to the second generation - the production of biofuels from cellulose (straw, leaves, shrubs, etc.), which is the most common biological material on Earth (Zinovieva *et al.* 2018, Demiray *et al.* 2021).

Plant biomass, including wood, having unique properties, structure and component composition, is an organic raw material, with proper influence on which it is possible to obtain both the most valuable chemical and feed products, and semi-finished products - pure wood components for the chemical industry (Hou *et al.* 2021). The use of modern highly efficient technological approaches in organizing these potentially demanded industries will significantly develop new technologies for the integrated processing of renewable plant materials (Danso *et al.* 2022).

One of the ways of practical application of this approach is the organization of an environmentally friendly process of high-temperature steam-explosion treatment, the resulting product of which is a lignocellulose fiber freed from hemicelluloses with a high specific surface area and reactivity (Sarker *et al.* 2021, Prosvirnikov *et al.* 2020, Prosvirnikov *et al.* 2021, Matsakas *et al.* 2018). The pre-activation of wood raw materials contributes to

---

* Corresponding author
Email: kulikova.pnipu@gmail.com (Y. Kulikova)

https://doi.org/10.61435/ijred.2026.61970

the acceleration of the delignification process, the reduction of the consumption of reagents and energy costs, the choice of more environmentally friendly reagents, the production of higher quality cellulose, the expansion of the range of wood species used in the delignification process, as well as accelerated acid and enzymatic hydrolysis.

In this regard, the present study is aimed at solving a scientific problem in the field of developing highly efficient technologies for deep chemical processing of wood waste and plant biomass using the method of preliminary steam explosive modification and obtaining valuable raw materials such as bioethanol.

The novelty of the proposed research lies in obtaining new fundamental knowledge about the influence of the characteristics of lignocellulose materials activated by the steam explosion treatment on the processes of their chemical and biochemical conversion to obtain an alternative energy resource - bioethanol. Understanding the processes and phenomena that occur during the chemical and biochemical conversion of such materials through enzymatic treatments and anaerobic fermentation to produce bioethanol will allow the development of improved, highly efficient technologies, the use of which in the energy, wood chemical, woodworking and agricultural industries in the foreseeable future seems to be very promising (Tohamy *et al.* 2019, Kharina *et al.* 2017, Akbarova *et al.* 2017, Boltovsky 2021).

This study contributes to sustainable energy development by offering an efficient method for converting agricultural and forestry waste into bioethanol. The application of steam explosion significantly enhances the reactivity of plant materials, making biofuel production faster and more productive. The proposed approach supports zero-waste principles by enabling the further use of by-products like pentose sugars and CO_2. The findings can be used to optimize industrial bioethanol production, especially in regions with abundant lignocellulosic residues. This technology provides an accessible alternative energy source, addressing both environmental challenges and rural economic needs.

2. Materials and methods

2.1 Raw Materials and Pre-treatment (Steam Explosion)

The raw material used in this study was pine wood waste (collected in the form of branches and twigs in the Kukmorsky district of the Republic of Tatarstan in the period August-September 2021), crushed in a hammer mill to the size of chips along the length of 5-15 mm, with an initial absolute humidity of 55 -60%. Also, the study used wheat straw, also collected in the Kukmorsky district of the Republic of Tatarstan in the period August-September 2021, crushed on a straw cutter to a size of 5-7 mm.

A pilot plant for processing activated agricultural plant waste into bioethanol was developed on the basis of the Department of Wood Materials Processing of the Federal State Budgetary Educational Institution of Higher Education "KNRTU". Activation of plant waste (wood, straw, husks of agricultural crops) is carried out by the method of steam explosion treatment (short-term exposure of plant raw materials to water steam followed by a sharp release of pressure, leading to chemical modification of the lignocellulose matrix and an increase in the specific surface area of the treated particles).

Work of installation has shown on the scheme (Figure 1). Shredded vegetable raw materials (wood waste, straw, crop husks) enter the cyclone 4 from the hopper 1 through the screw 2 and conveyor 3 and separated from the dust.

After separation, the raw material enters the impregnation chamber 5, where, depending on the raw material, acid catalysts are supplied from tanks 6 and 7. The impregnated and moistened raw material enters through the buffer tank 8 into the continuous steam explosion treatment chamber, where it is treated with saturated water vapor under pressure coming from the steam generator 10. After a short-term activation by steam hydrolysis, the raw material is “shot” into the blow tank 11 and washed with water from the degradation products of hemicelluloses (pentose sugars), from where it enters the hydrolysis boiler 14 in the form of an activated mass through the screw 13, in which it is possible to carry out both acid hydrolysis with weak solutions acids, and enzymatic hydrolysis using cellulase preparations. The hydrolyzing agent is circulated by pump 16 and heated if necessary. After complete hydrolysis of cellulose to monosaccharides, the hydrolysate is neutralized and pumped through the filter pump 17 to the fermenter 18, and the remaining lignin is blown with steam into the reservoir 11. In the apparatus 18, the neutralizate is cooled and settled. Further, the prepared neutralizate is enriched with mineral components and filled with microorganisms for anaerobic digestion of reducing substances. At the end of alcoholic fermentation, the liquid alcohol-containing product is discharged from the apparatus 18 into the distillation column 21, the resulting carbon dioxide is discharged into the collector

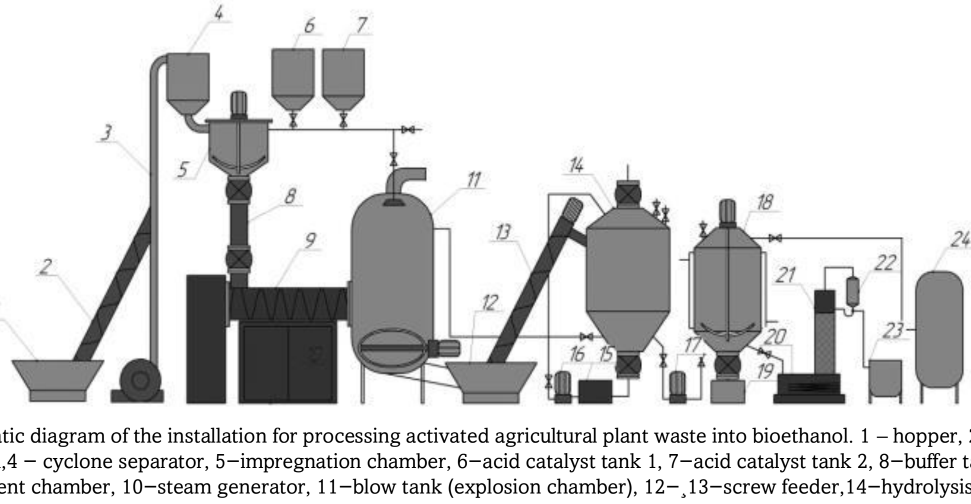


Fig. 1 Schematic diagram of the installation for processing activated agricultural plant waste into bioethanol. 1 – hopper, 2 – screw, 3 – conveyor main,4 – cyclone separator, 5–impregnation chamber, 6–acid catalyst tank 1, 7–acid catalyst tank 2, 8–buffer tank, 9– steam explosion treatment chamber, 10–steam generator, 11–blow tank (explosion chamber), 12–¸13–screw feeder,14–hydrolysis boiler (reactor), 15–, 16–circulation pump, 17–filter pump¸18–fermenter, 19–, 20–, 21–Distillation column,22– Condenser, 23–Ethanol collector ,24–CO_2 gas collector

24. The separation of alcohol occurs by evaporation in the lower part of the column 21 of the packed type. The vapors condense in the upper part and ethanol is collected in the form of a distillate in the collector 23.

2.2 The specific parameters for the "steam explosion"

Steam-explosive activation of raw materials was carried out on the installation presented above, at a temperature of saturated water vapor of 165 °C and 210 °C with a corresponding pressure of 0.6 and 1.9 MPa for 5 minutes. The raw material was preliminarily moistened to an absolute moisture content of 130-140% in an acetic acid-water medium in a mass ratio of 1:19 (5% solution) to accelerate the hydrolysis of hemicelluloses during steam explosion treatment.

Component analysis of the initial and steam-activated lignocellulose raw materials was carried out according to the following scheme: determination of cellulose content - by the chlorite method, lignin - by the sulfuric acid method, extractives - by the extraction method in the Soxhlet apparatus, difficult and easily hydrolyzed polysaccharides - by the Macen-Schoorl method (reducing agents).

2.2 Acid Hydrolysis Procedures

Wood raw materials, both in raw form and in activated form, were subjected to acid hydrolysis under the following regime parameters: H_2SO_4 concentration - 0.5% and 1.5%, hydromodulus 1:15, hydrolysis temperature - 187 °C, pressure - 1.1- 1.15 MPa, duration of hydrolysis - 5 hours (with the determination of the maximum of reducing agents - hereinafter RA). Samples of the hydrolyzate were taken every 30 minutes, the concentration of the total content of RA was determined in them by the Macken-Schoorl method, followed by recalculation of RA for absolutely dry raw materials.

2.4. Enzymatic Hydrolysis and Fermentation Conditions

Enzymatic hydrolysis was carried out for wheat straw without pre-treatment, and for straw activated by steam explosion treatment under the following regime parameters: enzyme preparation - Cellulox-A (in the form of a powder, JSC PO Sibbiopharm, Russia) (2000 units per 1 g of preparation) - 6 and 12 g/kg of raw material, hydrolysis temperature - 45 °C, substrate pH 4.7 (acetate buffer), raw material concentration in the substrate 33 g/l, hydrolysis duration 72 h, hydromodulus 1:5, mixing speed - 150 rpm /min. Samples of the hydrolyzate were taken every 8 hours, centrifuged for 6 min at 10,000 rpm, the concentration of RA and the component composition were determined similarly to acid hydrolysates.

Alcoholic fermentation of filtered and neutralized (in the case of acid hydrolysis) hydrolysates was carried out at a temperature of 32-34 °C using the yeast *Saccharomyces cerevisiae*, since they have a high productivity in terms of glucose, fructose, galactose, sucrose and they do not convert pentoses. Fermentation duration was 7 hours, yeast concentration - 25 g/l, water ratio - 1:10, mixing speed 80 rpm sampling every hour followed by distillation in a laboratory plant. Hydrolysates were used from the enzymatic hydrolysis of straw activated at 165 ⁰C and 210 ⁰C, as well as from the acid hydrolysis of pine wood activated at the same temperatures. In this study, steam explosion treatment was specifically carried out in order to maximize the reduction of pentoses in acid and enzymatic hydrolysates. The concentration of bioethanol was analyzed in the beer distillate using an ASP-1 hydrometer for alcohol. The yield of bioethanol in % of RA was recalculated after determining the mass yield.

2.5 Analytical Techniques and Equipment Characterization

The qualitative composition of dispersed and modified capillary-porous materials, including the presence and content of functional groups in activated lignocellulose samples, was evaluated using Fourier-transform infrared spectrometry on a Tensor 27 spectrometer (Germany) to obtain spectral characteristics of the components of dispersed capillary-porous lignocellulose material in order to measure the kinetics of reactions (cellulose, lignin, hemicelluloses, mainly pentosans). The spectra of the samples were recorded in reflection mode. KBr tablets were pressed for research purposes.

The component composition of the RA was determined by liquid chromatography on the Khromatek-Kristall 5000 hardware-software complex with a mass spectrometric detector. (the temperature of the isothermal operating mode of the column was 150 °C, the flow rate of carrier gas (nitrogen) was 60 cm^3/min, and the duration of chromatographic separation was 40-45 minutes). Since monosaccharides are non-volatile and it is impossible to directly separate their mixtures using gas-liquid chromatography, the sugars in the hydrolysates were modified into trimethylsilyl (TMS) derivatives of monosaccharides. These are volatile glycosides of simple O-trimethylsilyl esters of carbohydrates, which are formed by the complete substitution of the hydroxyl groups of monosaccharides during silylation. A mixture of trimethylchlorosilane and hexamethyldisilazane in a pyridine medium was used as the silylation reagent for monosaccharides.

The obtained chromatograms were processed by calculating the area of the peaks and determining the concentrations of monosaccharides in the hydrolysates (the total number of tautomeric forms of arabinose, xylose, mannose, galactose, and glucose). After determining the total amount of RA by the Macen-Schoorl method with additional oligosaccharide inversion, the amount of oligosaccharides was determined as the difference between the total amount of RA and the amount of monosaccharides determined by chromatography.

3. Results and discussion

Since the production of bioethanol from plant raw materials requires hydrolysates from hexose mono- and oligosaccharides, in this work, the raw materials were subjected to preliminary steam explosion treatment in a saturated water vapor environment. Any lignocellulosic raw material consists of cellulose, lignin and hemicelluloses, and the destruction of each of the components under different exposure conditions occurs in different ways. Thus, cellulose is hydrolyzed to simple sugars of the C6 group in the presence of weak acid solutions and elevated temperatures, or in concentrated acids at moderate temperatures. Lignin undergoes depolymerization both in the presence of acids and alkalis with the formation of complex low molecular weight condensed fragments. Hemicelluloses, as less stable polysaccharides, are hydrolyzed in the presence of weak acids, including organic ones, and are converted into mono- and oligosaccharides of the C5 group. Also, with prolonged exposure to temperature and catalysts, simple monosaccharides can further decompose to simple substances, for example, pentoses, as a rule, turn into furfural under harsh hydrolysis conditions, and the elimination of acetyl groups of xylans and mannans as a result of hydrolysis leads to the formation of organic acids, in particular acetic and formic. Thus, the latter phenomenon is used in the process of steam explosion treatment, in which acetic acid is formed, which is an additional

Table 1
Chemical composition of feedstock and activated lignocellulosic mass (ALM)

Substrate	Cellulose, % a.d.s.	Lignin, % a.d.s.	Hemicellulose, % a.d.s.
Pine wood (without treatment)	51.9±3.3	28.2±2.2	19.8±1.8
ALM (t_{st}=165 °C) from wood	62.11±4.6	26.8±2.0	11.0±1.3
ALM (t_{st}=210 °C) from wood	68.7±5.5	26.9±1.8	4.4±0.4

*ALM, activated lignocellulosic mass

catalyst for the hydrolysis of easily hydrolysable hemicellulose polysaccharides. Variation of steam explosion treatment temperature (further t_{st}) from 165 °C to 210 °C also contributes to the breakdown of hemicelluloses (Table 1) in any raw material to pentoses.

According to the data on the component composition of the substrates, an increase in the treatment temperature also contributes to a decrease in lignin, since during steam explosion treatment, lignin is partially depolymerized into soluble low-molecular-weight fragments, which are also removed during washing of the activated lignocellulosic mass (ALM). A slight decrease in the cellulose content (relative to the feedstock before processing) in terms of processing temperature is explained by the hydrolysis of the amorphous part of cellulose, into which weak organic acids penetrate faster than into the crystalline structure of the cell wall of the fibers. This is not reflected in Table. 1 since the data are given in mass % of an absolutely dry substrate after washing, that is, due to a decrease in the concentration of hemicelluloses and initial lignin, the proportion of cellulose in the final raw material increases.

Cellulose is the most stable component of the system. At this temperature, it does not degrade without the use of catalysts. The reduction in hemicellulose and lignin content led to a decrease in the dry residue of biomass, which in turn increased the relative cellulose content. This is a standard phenomenon observed in all studies on the thermal treatment of plant biomass. A similar trend can be seen in the works of Kälkäjä *et al.* 2025 and Kilpeläinen *et al.* 2012.

In the study by Kälkäjä *et al.* 2025, a two-step hot-water treatment was applied. The hemicellulose content decreased from 29.8% to 11.6%. The relative lignin content increased. After the second treatment step, a decrease in cellulose content was observed, which would subsequently reduce bioethanol yield. In our work, however, the absolute cellulose content remained unchanged.

Changes in the structures of pine fibers treated by the vapor-explosive method were also investigated by IR-Fourier spectrometry (Fig. 2). The results showed only some changes in the macromolecular structure of the fibers, which is obviously related to the multi-component nature of the material. The hydrophilicity of the treated and untreated samples was detected in a broad absorption band from 3500 cm^{-1} to 3300 cm^{-1}, which is associated with the -OH groups of the main components of lignocellulose. The peak at 2917 cm^{-1} is associated with the stretching vibrations of the aliphatic C-H bonds, which are shown by small changes in the absorption of the samples after vapor-explosive treatment. These changes are apparently related to the partial removal of lignin from the fibers. The two peaks at 1504 cm^{-1} and 1428 cm^{-1} correspond to the aromatic C-C bonds of the lignin aromatic ring.

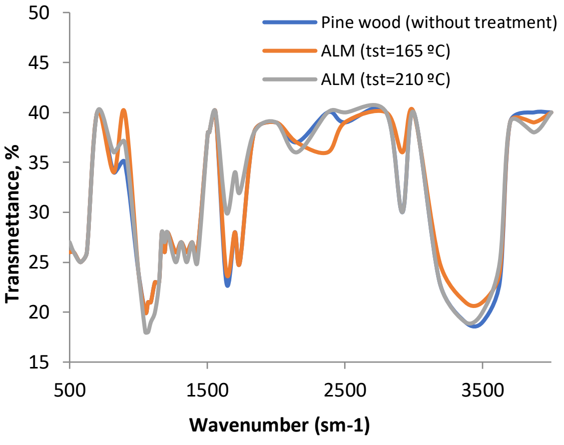


Fig. 2. IR spectra of pine wood samples: 1 - original, 2 - ALM at t_{st}=165 °C, 3 - ALM at t_{st}=210 °C

The characteristic peak at 1737 cm^{-1} in untreated fiber belongs to either the ester bonds of the acetyl and uronic groups of hemicelluloses, the ester bond of the carboxyl groups of hemicelluloses, or the ester bonds of the carboxyl groups of ferulic and p-coumaric acids of lignin. The intensity of this peak decreases in fibers treated with vapor-explosive activation at 210 °C. These processing conditions result in the removal of most of the hemicelluloses and some of the lignin from the fibers. The absorption at 1638 cm^{-1} is clearly related to the absorption of water. The decrease in the intensity of this peak in the processed aspen fiber is due to the partial removal of hemicelluloses. The absorptions at 1058 cm^{-1} and 896 cm^{-1} are associated with the stretching vibrations of the C-O bonds and the rocking vibrations of the C-H bonds in cellulose.

This is natural for both pine wood and wheat straw. In wheat straw, the component composition is similar to wood, but the straw contains a larger amount of ash and mineral impurities, which insignificantly contribute to the overall composition of the substrates after processing. Fig. 3 shows samples of processed and unprocessed plant material.

Thus, steam explosion treatment makes it possible to get rid of hemicelluloses and pentose sugars, which do not contribute to the formation of ethanol during alcoholic fermentation, while the yield of reducing substances (mainly C6 monosaccharides) increases due to this. It should also be noted that after the steam explosion treatment of high-moisture plant raw materials, which is produced under excessive pressure, the raw material acquires a developed specific surface due to pressure relief and explosive boiling of the liquid inside the fibers of the raw material. This leads to separation of the fibers from each other, which increases the accessibility of the cell wall to hydrolysing agents, both acidic and enzyme preparations. Together with the chemical transformations of the components, the developed specific surface increases the hydrolysis reactivity of the substrates by several times, which is the main advantage of the steam explosion treatment.

As mentioned earlier, wood raw materials (untreated and activated by steam explosion treatment) were subjected to acid hydrolysis in the presence of dilute sulfuric acid (0.5 and 1.5%) at a temperature of 187 °C. Figure 4 shows the data on the yield of reducing substances in mass % of the absolutely dry substrate. The black lines show the results of hydrolysis in 1.5% H_2SO_4, the red lines show the results of hydrolysis in 0.5%

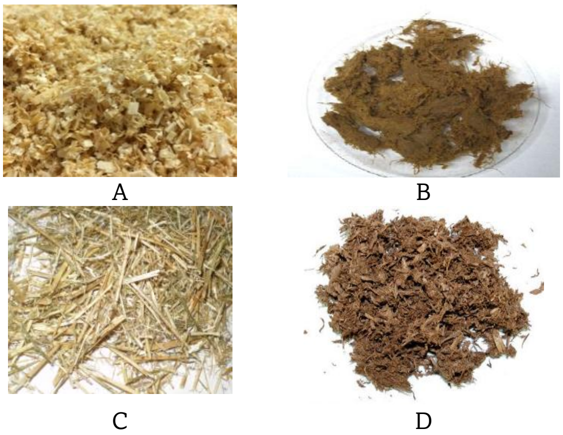


Fig. 3 Samples of processed and unprocessed plant material. A – Chopped pine wood; B – ALM from pine wood (t_{st}=210 °C); C – Crushed wheat straw; D – ALM from wheat straw (t_{st} =165 °C) *ALM, activated lignocellulosic mass

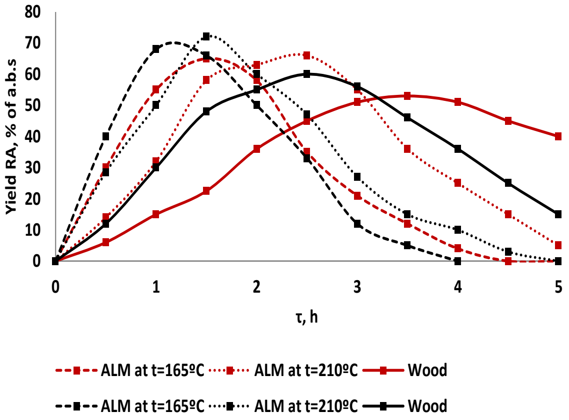


Fig. 4 Kinetics of the release of RA from wood raw materials during acid hydrolysis in 1.5% and 0.5% H_2SO_4, % a.d.s. The black lines show the results of hydrolysis in 1.5% H_2SO_4; The red lines show the results of hydrolysis in 0.5% H_2SO_4. *ALM, activated lignocellulosic mass

H_2SO_4. As can be seen from the presented data, all curves reach a certain maximum of RA (monosaccharides) in time, after which their content decreases, since the resulting monosaccharides further decompose into simpler substances. Therefore, the optimal duration of hydrolysis is the time at which the maximum yield of RA is observed.

The maximum theoretical yield of RA in wood according to the raw materials is 71.7% (polysaccharides of cellulose and hemicelluloses). In practice, a maximum yield of 59.9% (83.5% of the theoretical yield) is observed after 2.5 h of hydrolysis in 1.5% H2SO4, and 53% (73.9% of the theoretical yield) after 3.5 h during hydrolysis of 0.5% H2SO4. In general, for the case of wood, this is a comparable result, especially in time, since untreated wood particles have a high density, pores are partially filled with air, which makes it difficult for the reagent to penetrate the structure of the cell wall. Increasing the acid concentration increases the maximum output of RA for wood by 13% and reduces the hydrolysis time by an average of 1 hour. Activated wood subjected to hydrolysis is most efficiently converted into RA within 1-1.5 hours after the start of hydrolysis, which is 1.7-2.5 times faster compared to untreated wood. Obviously, this is due to the high reactivity of ALM, with more than 95% of the maximum yield of RA from ALM being achieved. Increasing the sulfuric acid concentration from 0.5% to 1.5% raises the concentration of hydronium ions (H_3O^+), which play a key role in the mechanism of acid-catalyzed cellulose hydrolysis. As a result, the hydrolysis efficiency improves. However, carrying out the process at higher sulfuric acid concentrations may lead to the formation of undesirable by-products.

Chromatograms for pine wood and straw hydrolysates are presented in Supplementary materials - Figures 1S (a-c) and 2 S (a-c) accordingly. It should also be noted that RA obtained from ALM predominantly (up to 90%) consist of hexoses, in contrast to RA from wood, the sum of which consists of approximately 70% hexose monosaccharides and 30% pentoses. Thus, reducing the time of ALM hydrolysis to the maximum yield of RA is a significant influencing factor on the economic justification for the process of obtaining bioethanol from plant materials activated by steam explosion treatment.

Enzymatic hydrolysis was carried out both for straw without pretreatment and for ALM from straw. Fig. 4 shows the results of the enzymatic hydrolysis of this raw material. The black lines show the yield of RA with 12 g of the enzyme preparation per 1 kg of substrate; the red lines show 6 g/kg of substrate. As can be seen from Fig. 5, untreated straw is weakly hydrolyzed in the presence of a cellulase complex and reaches 10-16.6% of the RA from the mass of the initial substrate (with a theoretically possible 48.7%). Increasing the consumption of the enzyme preparation to 12 g/kg of the substrate increases the total yield of RA by only 6.6%. The depth of the enzymatic hydrolysis of straw significantly increases after the preliminary steam explosion activation of polysaccharides, while the yield of RA reaches 56.6-68.3% of the initial content of polysaccharides in the raw material. The highest yield of RA (68.3%) is observed for straw activated at 165 °C (at the consumption of the enzyme preparation 12 g/kg of the substrate). The reduced consumption of the enzyme preparation makes it possible to obtain 60% RA for this raw material at 72 h of hydrolysis. It is noticeable that an increase in the temperature of steam explosion treatment to 210 °C reduces the yield of RA by about 5.7-7.3%, depending on the consumption of the enzyme preparation.

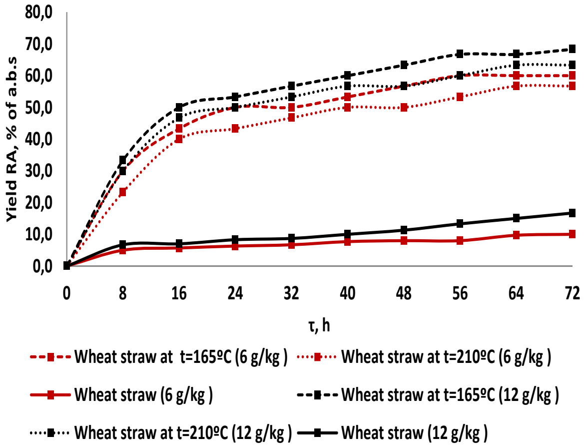


Fig. 5 Kinetics of the release of RA from non-wood raw materials (wheat straw without treatment and after steam explosion treatment at temperatures Tpo = 165 and 210 °C) during enzymatic hydrolysis at the expense of the enzyme preparation of 6 and 12 g/kg of substrate The black lines show the yield of RA with 12 g of the enzyme preparation per 1 kg of substrate; The red lines show 6 g/kg of substrate.*RA, reducing agents

At temperatures of steam explosion treatment above 200 °C, degradation products of monosaccharides and organic acids, furan derivatives and terpenes are formed, which negatively affect the activity of enzyme preparations, which leads to a decrease in their productivity and a reduced yield of RA. Thus, the influence of the destroyed cell wall in ALM and its high reactivity as a result of steam explosion treatment significantly affects the rate of enzymatic lysis. A comparative analysis of acid and enzymatic hydrolysates (Table 2) showed that, in addition to RA, the composition of hydrolysates also includes other products of polysaccharide hydrolysis - uronic acids, furfural, organic acids, degradation products of lignin and extractives.

The most important components of hydrolysates are monosaccharides. Their total content in the hydrolysates of untreated pine is 3.61% (of which sugars of the C6 group are 2.8%, C5 are 0.61%), and in the hydrolysates of ALM pine from 4.08 (of which sugars of the C6 group are 3, 38%, C5 - 0.7%) to 4.31% (of which group C6 sugars - 4.25%, C5 - 0.06%). It should be noted that there are practically no oligosaccharides in ALM hydrolysates, which indicates a fairly high depth of hydrolysis, which cannot be said about the hydrolyzate from wood, in which the remaining oligosaccharides must be subjected to inversion (additional hydrolysis to monosaccharides). The total content of monosaccharides in hydrolysates of untreated straw is only 0.5% (of which group C6 sugars - 0.28%, C5 - 0.01%), and in straw ALM hydrolysates from 1.78 (of which group C6 sugars - 1, 49%, C5 - 0.18%) to 1.69% (of which group C6 sugars - 1.61%, C5 – not detected). Straw hydrolysates also show a high content of oligosaccharides, but they are characterized by a lower content of pentose sugars compared to wood.

As can be seen from the presented spectra, with an increase in the temperature of vapor-explosive processing, the amount of pentose monosaccharides (arabinose, xylose) decreases and at 210 ºC is practically absent (peaks from 2 to 16 min), and in all tautomeric forms. This is a direct indication of the destruction of C5 group monosaccharides with an increase in temperature, which affects the quality of the hydrolysate for further biochemical processing.

From the above data, we can conclude that the most acceptable and benign hydrolysates from pine are obtained by activating it by the steam explosion method at a temperature of 210 °C, hydrolysis of ALM in the presence of 1.5% H_2SO_4, which will make it possible to obtain 4.31% RV with a minimum content of pentoses. As for straw, it is more expedient to carry out enzymatic hydrolysis at an EP consumption of 12 g/kg also with ALM obtained at 210 °C, which will make it possible to obtain hydrolysates with a content of 1.69% RA with a minimum content of pentoses and oligosaccharides.

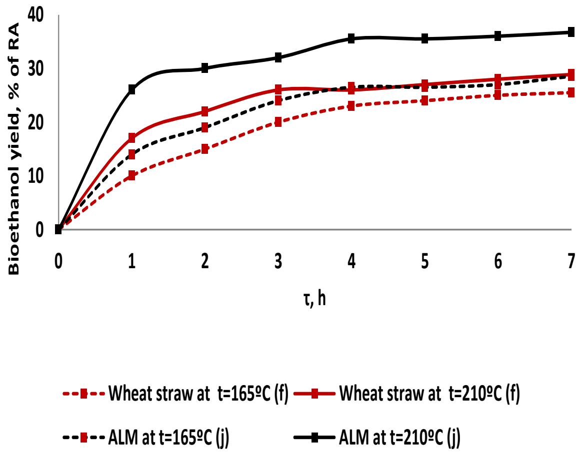


Fig. 6 Kinetics of bioethanol yield in mass% of the concentration of reducing agents in hydrolysates *ALM, activated lignocellulosic mass; RA, reducing agents

During alcoholic fermentation, hexose monosaccharides are transformed under anaerobic conditions by their incomplete oxidation, while the release of thermal energy is observed. Enzymatic transformations of monosaccharides occur inside the cells of microorganisms in the presence of the necessary inorganic nutrients. Through the semi-impermeable shell of the yeast cell, metabolic products are released into the wort - carbon dioxide, ethanol, as well as some by-products of the biochemical process. In a study on the fermentation of prepared and purified enzyme and acid hydrolysates obtained from steam-activated plant materials, the yeast *Saccharomyces cerevisiae* was used, since they have a high productivity for glucose, fructose, galactose, sucrose and they do not convert pentoses. Fig. 6 presents data on the alcoholic fermentation of the resulting hydrolysates. As can be seen from Fig. 6, the highest yield of ethanol is observed with the conversion of the pine ALM hydrolyzate activated at a temperature of 210 °C and

Table 2
Component composition of hydrolysates

Component, % in hydrolyzate	Pine, 1.5% (C)	ALM of pine, 165 °C, 1.5% H_2SO_4	ALM of pine, 210 °C, 1.5% H_2SO_4,	Wheat straw	ALM of straw 165 °C	ALM of straw 210 °C
				enzyme dose 12 g/kg		
D-glucose	2.0±0.1	2.95±0.11	2.8±0.2	0.11±0.1	1.15±0.058	1.43±0.072
D-galactose	0.10±0.01	0.12±0.01	0.35±0.03	0.15±0.1	0.083±0.004	0.093±0.005
D-mannose	0.7±0.1	0.31±0.02	1.1±0.1	0.022±0.002	0.26±0.02	0.094±0.006
D-xylose	0.45±0.03	0.46±0.04	0.054±0.002	0.011±0.001	0.16±0.01	0
L-arabinose	0.16±0.01	0.39±0.03	0.013±0.001	0	0.022±0.001	0
Oligo- and polysaccharides	0.02±0.02	0.056±0.005	0	0.21±0.01	0.11±0.01	0.083±0.006
Uronic acids	0.02±0.01	0.016±0.02	0	0.048±0.003	0.034±0.02	0.014±0.002
Furfural	0.12±0.01	0.21±0.01	0.34±0.02	0.022±0.002	0.18±0.01	0.24±0.01
5-hydroxymethylfurfural	0.18±0.02	0.28±0.02	0.51±0.03	0.015±0.002	0.33±0.02	0.51±0.03
Levulinic acid	0.22±0.02	0.012±0.002	0	0.012±0.003	0.052±0.009	0
Formic acid	0.033±0.003	0	0	0	0.18±0.02	0
Acetic acid	0.41±0.02	0.12±0.01	0.14±0.01	0.07±0.01	0.15±0.01	0.054±0.003
The total yield of RA in the hydrolyzate, %	3.61±0.09	4.08±0.18	4.31±0.21	0.51±0.02	1.78±0.07	1.69±0.08
Total organic matter in the hydrolyzate, %	4.59±0.08	4.76±0.07	5.26±0.06	0.65±0.03	2.62±0.11	2.50±0.12

Table 3
Influence of biomass pretreatment on bioethanol production

Raw material	Pretreatment parameters	Yield (%)	Reference
Wheat straw	Steam explosion + enzymatic hydrolysis	28.6	Experimental data
Wood Sawdust	Steam explosion + acid hydrolysis	36.7	Experimental data
Pine needles	1% NaOH, 90°C, 5 h	15-16	Wawro *et al.* 2023
Wheat straw	1% NaOH, room temperature, 24 h	2	Sharma *et al.* 2021
Wheat straw	0.5% H_2SO_4, 2% H_3PO_4, 150–200°C, solid-to-liquid ratio 1:20, 1–10 min	58.82 и 88.24	Marđetko *et al.* 2018
Wheat straw	Enzymatic hydrolysis	7	Novy *et al*, 2015
Wheat straw	Steam explosion, 170–200°C, 10–20 min	10	Ballesteros *et al.* 2006

it accounts for 36.7% of the RA. About 28.6% of ethanol can be obtained after 7 hours of biochemical process on the ALM hydrolyzate of pine, activated at a temperature of 165°C. Since the last hydrolyzate contains almost 1% more RA of pentose monosaccharides and oligosaccharides in its composition (Table 2), a reduced ethanol yield on such a hydrolyzate is expected.

A similar picture is observed for activated straw hydrolysates, however, the difference in ethanol yield is not as significant as in the case of pine, and amounts to 11.6% at the maximum point, nevertheless, it is present. The maximum yield of ethanol during the processing of straw can be obtained by first activating it by steam explosion treatment at a temperature of 210 °C. Although at this activation temperature, the hydrolysate contains less sugars (1.69% vs. 1.78% at 165 °C), these hydrolysates have virtually no pentoses and oligosaccharides, while the ethanol yield was higher.

From the two examples, it can be concluded that the increased content of pentoses and oligosaccharides in the hydrolysate is a limiting inhibitory factor affecting the vital activity of the yeast *Saccharomyces cerevisiae*, and as a result, leads to a lower ethanol yield. It is worth noting that at higher activation temperatures, more Furfural and 5-hydroxymethylfurfural are converted into hydrolysate, as shown in Table 2, but these components did not act as inhibitors of yeast *Saccharomyces cerevisiae*, at least in this study.

This may be due to the fact that during the adaptation phase, *Saccharomyces cerevisiae* cells rapidly decompose furfural in solution, producing furfuryl alcohol as the main product. Sroka *et al.* 2023 showed that low concentrations of furfural had a beneficial effect on yeast adaptation processes or cellular metabolism aimed at reducing furfural to furfuryl alcohol, while higher concentrations of furfural did not significantly affect the fermentation rate or the main parameters of the fermented wort. It is clear that the main inhibitory factors in fermentation processes are the concentrations of sugars, especially C5 groups and oligosaccharides, as well as the pH of the medium.

Since the conversion to bioethanol involves in hexose monosaccharides, the most effective hydrolysates obtained from the hydrolysis of steam explosive activated plant materials proved to be the most effective. Table 3 provides a comparative analysis of bioethanol production technologies in relation to the approach used in the present work. The data presented in the Table 3 show that the yield of bioethanol using steam-explosive activation (in this work) allows increasing the yield of ethanol from wheat straw by 4 times compared to the use of simple hydrolysis with enzymes. Steam activation of pine wood allows for a 2.5-fold increase in ethanol yield compared to processes using hydrolysates obtained by chemical hydrolysis.

A review of the literature indicates that acid hydrolysis of cellulose enables the most efficient sugar-to-bioethanol conversion. In contrast, alkaline pretreatment yields lower product output, likely because it maintains cellulose crystallinity. Although our approach underperforms relative to the acid hydrolysis process described by Marđetko. (2018) — potentially due to unaccounted variables — the proposed technology remains competitive and suitable for industrial bioethanol manufacturing.

4. Conclusion

Based on the presented results, it can be concluded that lignocellulosic material activated by the steam explosion method demonstrates high efficiency in enzymatic hydrolysis and subsequent biochemical conversion of the resulting hydrolysates into bioethanol. Activated wood reaches maximum reducing agents (RA) yield within 1–1.5 hours after the start of hydrolysis, which is 1.7–2.5 times faster compared to untreated wood.

The optimal temperature for steam explosion activation was determined to be 210 °C. At this temperature, the bioethanol yield reaches:

- From wood — up to 200 kg (253 L) of ethanol per 1 ton of absolutely dry wood, or 0.26 kg (0.33 L) of ethanol (36.7% of RA) per 1 kg of activated lignocellulosic mass (ALM);
- From straw — up to 140 kg (177 L) of ethanol per 1 ton of absolutely dry straw, or 0.172 kg (0.218 L) of ethanol (28.6% of RA) per 1 kg of treated straw.

The most effective pretreatment method for wood is steam explosion activation followed by acid hydrolysis in the presence of 1.5% H_2SO_4, while for straw it is steam explosion activation followed by enzymatic hydrolysis. The conducted research aligns with global trends in the conversion of plant raw materials into energy resources. Given the situation in Russia regarding the inefficient processing of plant and agricultural waste, as well as the high demand for affordable alternative energy sources with high added value at enterprises processing low-grade plant materials, this research direction is highly relevant.

Author Contributions: D.P.: contributed to conceptualisation, project leadership, data analysis, writing – the initial draft, writing – revisions. D.T.: responsible for methodology, data collection, and project management. Y.K.: data analysis, and writing – the initial draft. O.B.: validation; funding acquisition.

Funding: This work was supported by Ministry of Science and Higher Education of the Russian Federation within the framework of the project for the creation and operation of the Rosyanka carbon testing site in the Kaliningrad region (project FZVM-2024-0014)-other investigations.

Conflicts of Interest: The authors declare no conflict of interest.

References

Akbarova, N.A., Agzamova, N.A., Dzhahangirova, G.Z. (2017) Investigation of a method for producing bioethanol from nontraditional raw materials. *Universum: technical sciences*, 8(41), 42-46; URL: https://cyberleninka.ru/article/n/issledovanie-sposoba-polucheniya-bioetanola-iz-netraditsionnogo-syrya

Ballesteros, I., Negro, M. J., Oliva, J. M., Cabañas, A., Manzanares, P., & Ballesteros, M. (2006). Ethanol production from steam-explosion pretreated wheat straw. *Applied biochemistry and biotechnology*, 129-132, 496–508. https://doi.org/10.1385/abab:130:1:496

Boltovsky, V.S. (2021) Enzymatic hydrolysis of plant raw materials: state and prospects. *Proceedings of the National Academy of Sciences of Belarus, Chemical Series*, 57(4), 502-12. https://doi.org/10.29235/1561-8331-2021-57-4-502-512

Burdukov, A., Lomovsky, O.I., Bychkov, A.L., Chernetskiy, M.Y., Chernova, G.V. (2018). The effective use of straw crops as feedstock for the complex processing into biofuel. *Journal of the Siberian Federal University. Engineering & Technologies*, 11(2), 229–241. https://doi.org/10.17516/1999-494X-0026

Danso, B., Ali, S.S., Xie, R., Sun, J. (2022) Valorisation of wheat straw and bioethanol production by a novel xylanase- and cellulase-producing Streptomyces strain isolated from the wood-feeding termite, Microcerotermes species. *Fuel*, 310, 122333. https://doi.org/10.1016/j.fuel.2021.122333

Demiray, E., Kut, A., Karatay, S.E., Dönmez, G. (2021) Usage of soluble soy protein on enzymatically hydrolysis of apple pomace for cost-efficient bioethanol production. *Fuel*, 289, 119785. https://doi.org/10.1016/j.fuel.2020.119785

Hou, J., Zhang, X., Zhang, S., Wang, K. (2021) Enhancement of bioethanol production by a waste biomass-based adsorbent from enzymatic hydrolysis. *Journal of Cleaner Production*, 291, 125933. https://doi.org/10.1016/j.jclepro.2021.125933

Kälkäjä, S., Hu, T., Rusanen, A., Kärkkäinen, J., Lappalainen, K. (2025). Sequential utilization of birch sawdust using a two-step hot water treatment. *Biomass Conversion and Biorefinery*, 15, 10411–10423. https://doi.org/10.1007/s13399-024-05946-y

Keldiyorova, Sh., Toshmurodov, D., Alikulov, B. (2018) Review of modern research on enzymatic hydrolysis of lignocellulose-containing raw materials. *Bulletin of Science*, 1(3), 96–102. URL: https://www.вестник-науки.рф/article/2876

Kharina, M.V., Grigorieva, O.N. (2017) Design features of reactors for acid hydrolysis of lignocellulose-containing raw materials. *Bulletin of Kazan Technological University*, 20(13), 143–150. URL: https://cyberleninka.ru/article/n/osobennosti-konstruktsii-reaktorov-dlya-kislotnogo-gidroliza-lignotsellyulozosoderzhaschego-syrya

Kilpeläinen, P., Leppänen, K., Spetz, P., Kitunen, V., Ilvesniemi, H., Pranovich, A. & Willför, S. (2012). BIOREFINERY. Pressurised hot water extraction of acetylated xylan from birch sawdust. *Nordic Pulp & Paper Research Journal*, 27(4), 680-688. https://doi.org/10.3183/npprj-2012-27-04-p680-688

Lakina, N.V., Doluda, V., Rabinovich, G., Doluda, E., Lakina, M. (2021). Study of methods of processing biomass for the purpose of obtaining bioethanol. *Bulletin of Science and Practice*, 4(12), 96–100. https://doi.org/10.5281/zenodo.2254760

Marđetko, N., Novak, M., Trontel, A., Grubišić, M., Galić, M., Šanteka, B. (2018). Bioethanol Production from Dilute-acid Pre-treated Wheat Straw Liquor Hydrolysate by Genetically Engineered Saccharomyces cerevisiae. *Chemical and Biochemical Engineering Quarterly*, 32(4), 483-499 https://doi.org/10.15255/CABEQ.2018.1409

Matsakas, L., Nitsos, C., Raghavendran, V., Yakimenko, O., Persson, G., Olsson, E., Rova, U., Olsson, L., Christakopoulos, P. (2018) A novel hybrid organosolv: steam explosion method for the efficient fractionation and pretreatment of birch biomass. *Biotechnology for Biofuels and Bioproducts*, 11(1), 1–14. https://doi.org/10.1186/s13068-018-1163-3

Novy, V., Longus, K. & Nidetzky, B. (2015). From wheat straw to bioethanol: integrative analysis of a separate hydrolysis and co-fermentation process with implemented enzyme production. *Biotechnology for Biofuels*, 8, 46 https://doi.org/10.1186/s13068-015-0232-0

Prosvirnikov, D.B., Timerbaev, N.F., Sattarova, Z.G. (2020) Strength properties of composite board materials based on ligno-cellulose fiber, modified by steam treatment explosion. *Solid State Phenomena*, 299, 986–92. https://doi.org/10.4028/www.scientific.net/SSP.299.986

Prosvirnikov, D.B., Safin, R.R., Kozlov, R.R. (2021) Evaluation of the Influence of the Conditions of Catalytic Continuous Steam Explosive Activation of Wood on the Physical and Operational Properties of Wooded Composite Materials Based on Activated Fibers. *Key Engineering Materials*, 887, 129–37. https://doi.org/10.4028/www.scientific.net/KEM.887.129

Sarker, T.R., Pattnaik, F., Nanda, S., Dalai, A.K., Meda, V., Naik, S. (2021) Hydrothermal pretreatment technologies for lignocellulosic biomass: A review of steam explosion and subcritical water hydrolysis. *Chemosphere*, 284, 131372. https://doi.org/10.1016/j.chemosphere.2021.131372

Sharma, S., Jha, P.K. & Panwar, A. (2021). Production of bioethanol from wheat straw via optimization of co-culture conditions of Bacillus licheniformis and Saccharomyces cerevisiae. *Discover Energy*, 1, 5 https://doi.org/10.1007/s43937-021-00004-4

Sinitsyn, A.P., Sinitsyna, O.A. (2021) Bioconversion of renewable plant biomass by the example of second-generation biofuel: raw materials, preprocessing, enzymes, processes, economics. *Advances in Biological Chemistry*, 61, 166–195. https://doi.org/10.1134/S0006297921140121

Sroka, P., Tarko, T., & Duda, A. (2023). The Impact of Furfural on the Quality of Meads. *Molecules*, 29(1), 29 https://doi.org/10.3390/molecules29010029

Tohamy, E.Y., El-Gamal, A.D., Abouelwafa, A.M. (2019) Bioconversion of rice straw into bioethanol by enzymatic hydrolysis of Bacillus subtilis. I*OSR Journal of Pharmacy and Biological Sciences*, 14, 9–29. https://doi.org/10.9790/3008-1404040929

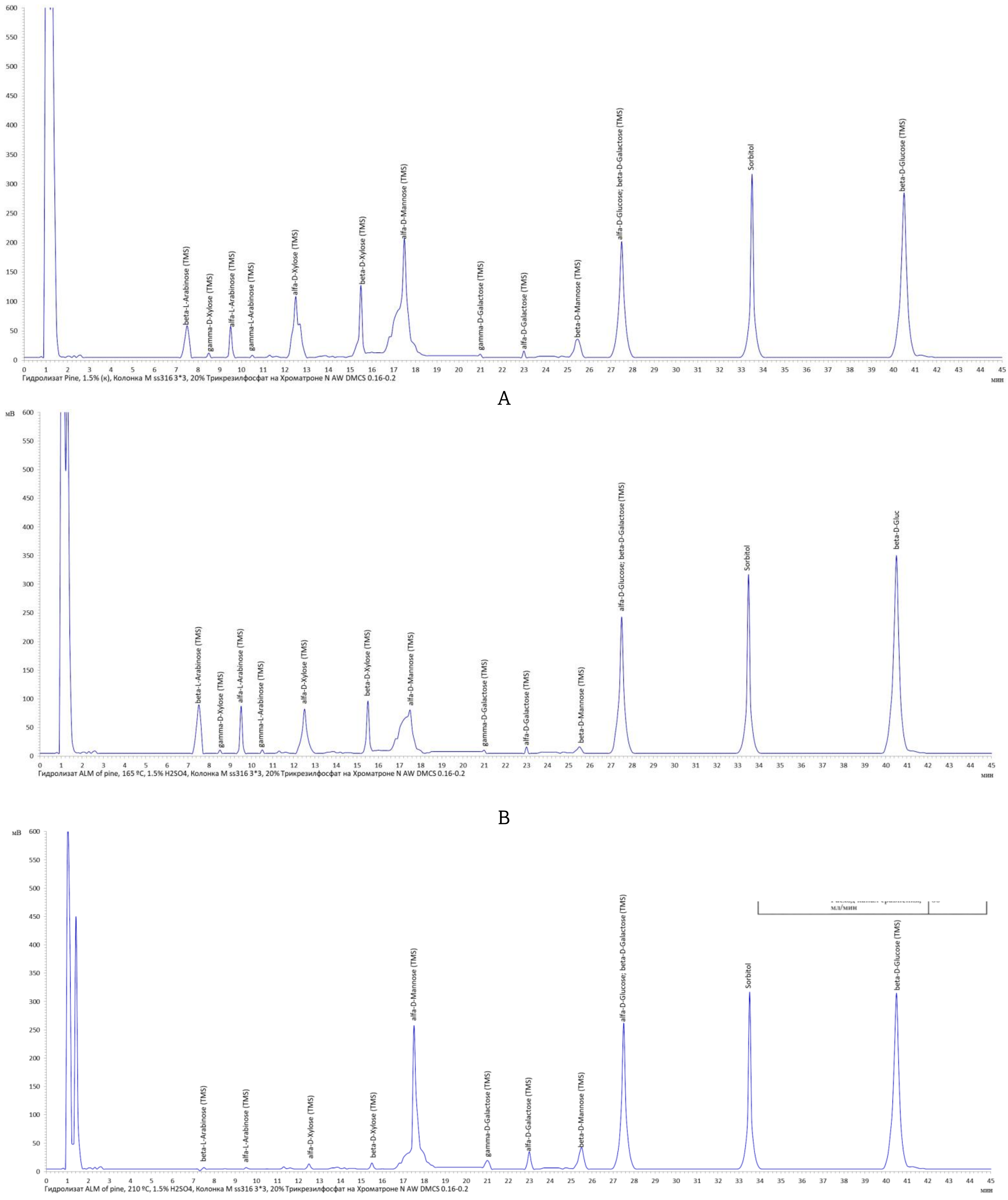
Tsegaye, B., Balomajumder, C., Roy, P. (2019) Alkali pretreatment of wheat straw followed by microbial hydrolysis for bioethanol production. *Environmental technology*, 40(9), 1203–1211. https://doi.org/10.1080/09593330.2017.1418911

Wawro, A., Jakubowski, J., Gieparda, W., Pilarek, Z., Łacka, A. (2023). Potential of Pine Needle Biomass for Bioethanol Production. *Energies*, 16(9), 3649 https://doi.org/10.3390/en16093949

Zinovieva, M.E., Volkova, T.S., Shafigullina, N.F. (2018) Features of enzymatic hydrolysis of cellulose-containing raw materials by the enzyme preparation "Cellolux-A". *Bulletin of the Technological University*, 21(3), 56–8. URL: https://www.elibrary.ru/item.asp?edn=xmjwnv

Appendix.

Supplementary materials



A

B

C

Fig. 1S Chromatograms of the monosaccharide composition of pine hydrolysates: a) pine (acid hydrolysis), b) ALM pine at 165 ⁰C (acid hydrolysis), c) ALM pine at 210 ⁰C (acid hydrolysis)

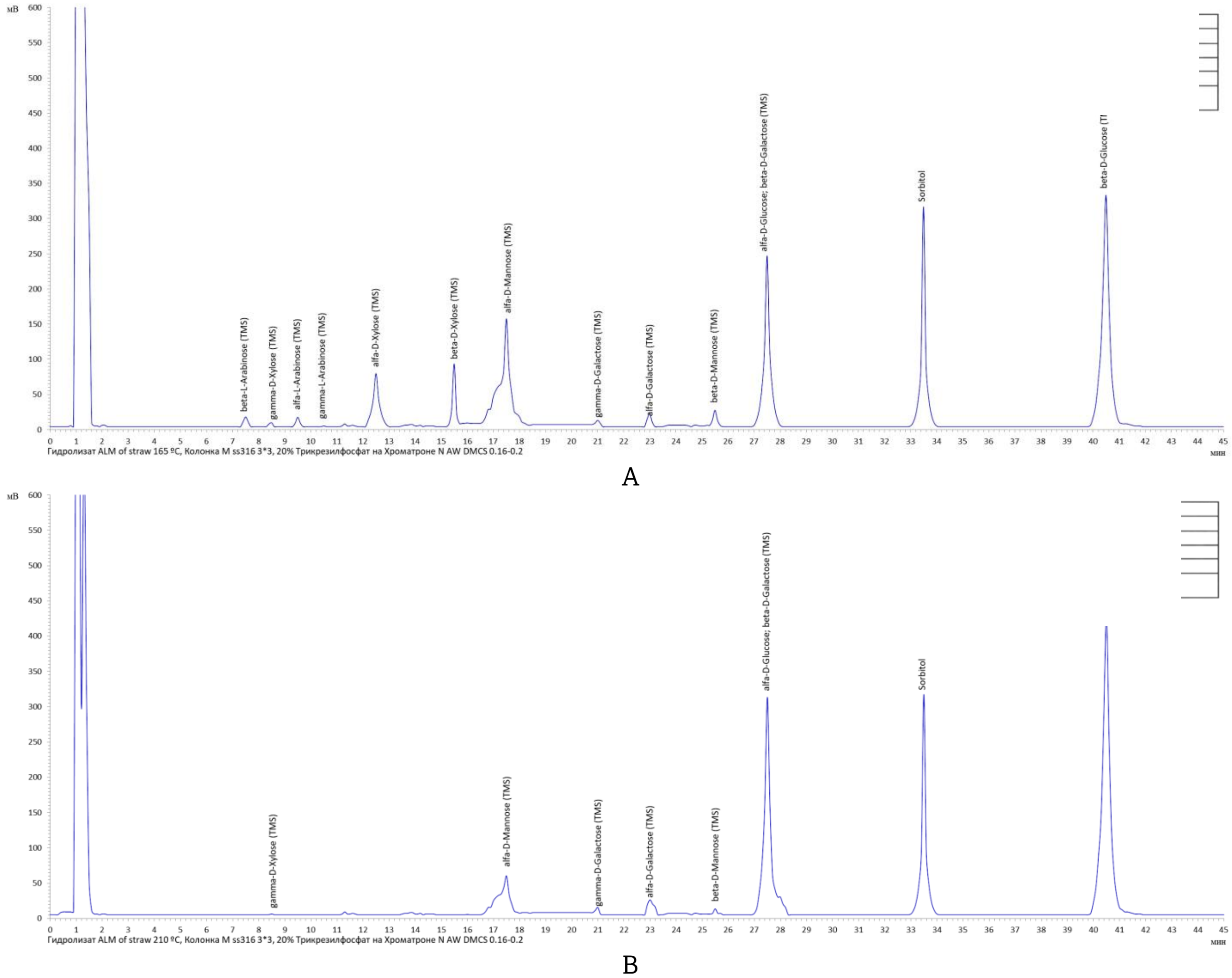


Fig. 2S Chromatograms of the monosaccharide composition of straw hydrolysates: a) straw (enzymatic hydrolysis), b) ALM straw at 165 °C (enzymatic hydrolysis), c) ALM straw at 210 ºC (enzymatic hydrolysis)